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13. ABSTRACT (Maximum 200 Words)

NKX3.1 is a homeoprotein with prostate-specific expression in adults. Loss of NKX3.1 correlates with prostate cancer progression. NKX3.1 protein expression is reduced to varying degrees in virtually all primary prostate cancers. The *NKX3.1* gene is affected by deletion and/or promoter hypermethylation in 90% of primary prostate cancers. NKX3.1 acts as a transcription factor by binding directly to DNA. NKX3.1 also complexes and coactivates other transcription factors such as serum response factor. We have now found that NKX3.1 complexes with the DNA unwinding enzyme topoisomerase I. NKX3.1 binds to topoisomerase I in a stoichiometric relationship and enhances scissile strand DNA cleavage by topoisomerase I. NKX3.1 does not affect religation of relaxed DNA by topoisomerase I. We also found that NKX3.1 mediates DNA damage repair after cells are exposed to γ-irradiation. The effect on DNA repair is mediated in cooperation with topoisomerase I. Loss of NKX3.1 expression that occurs early in prostate cancer may predispose to DNA damage and thereby facilitate prostate cancer progression.

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Introduction

NKX3.1 is a prostate-specific suppressor homeoprotein affected by haploinsufficiency that is the gatekeeper event for a large fraction of human prostate cancer. The *NKX3.1* gene maps to chromosome 8p21 which is lost in up to 85% of prostate cancer samples (1). Haploinsufficiency of *NKX3.1* results in an average reduction in intracellular protein expression of 30%, but as much as 70% in some tumor cells (Asatiani et al, *Cancer Research,* in press). The effect of haploinsufficiency on expression levels in human prostate cancers is comparable to the reduction in Nkx3.1 protein levels in gene-targeted *Nkx3.1*$^{+/-}$ mice that, with longer latency, develop prostatic hyperplasia and dysplasia similar to *Nkx3.1*$^{-/-}$ mice (2). *Nkx3.1* heterozygosity in mice augments the effect of other targeted oncogenic mutations on prostate cancer formation (3, 4). Important for the argument that *NKX3.1* loss of function is an initiating event in the development of human prostate cancer is the finding that protein expression levels in human high grade PIN are reduced comparable to levels in adjacent invasive cancer (Asatiani et al, C*ancer Research*, in press). Further support for the effect of haploinsufficiency on prostate cancer predisposition comes from the recent identification of a prostate cancer family with an inactivating mutation in the NKX3.1 homeodomain (W. Isaacs and J. Xu, personal communication).

NKX3.1 has a complex function that includes binding directly to DNA via the homeodomain resulting, most commonly, in transcriptional suppression. NKX3.1 also binds to other transcription factors such as serum response factor, also via the NKX3.1 homeodomain. In this case NKX3.1 enhances transcription of SRF-responsive genes.

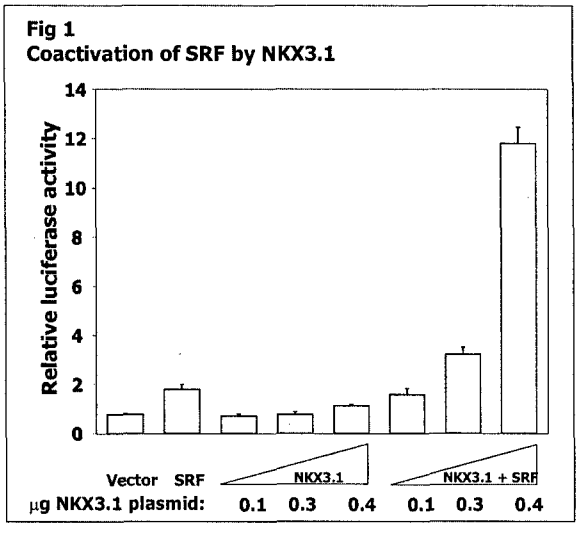
This project was aimed at two critical goals. The first was to identify a reporter that was sensitive to activation by NKX3.1. To this end we have now identified three promoter cassettes that are activated by NKX3.1. The second part of this project was to identify proteins that complexed with NKX3.1 and to explain the functional significance of the interaction between these proteins and both normal and polymorphic NKX3.1.

A. Construction and testing of NKX3.1 reporter genes with wild-type and mutant *NKX3.1* expression vectors. (Year 1)

Our lab has devised a number of assays to analyze the effect of NKX3.1 on transcription in addition to SRF coactivation. The CMV promoter interaction and IGFBP3 promoter activation are both independent of SRF and represent unique activities of NKX3.1 whose mechanisms remain to be fully explained.

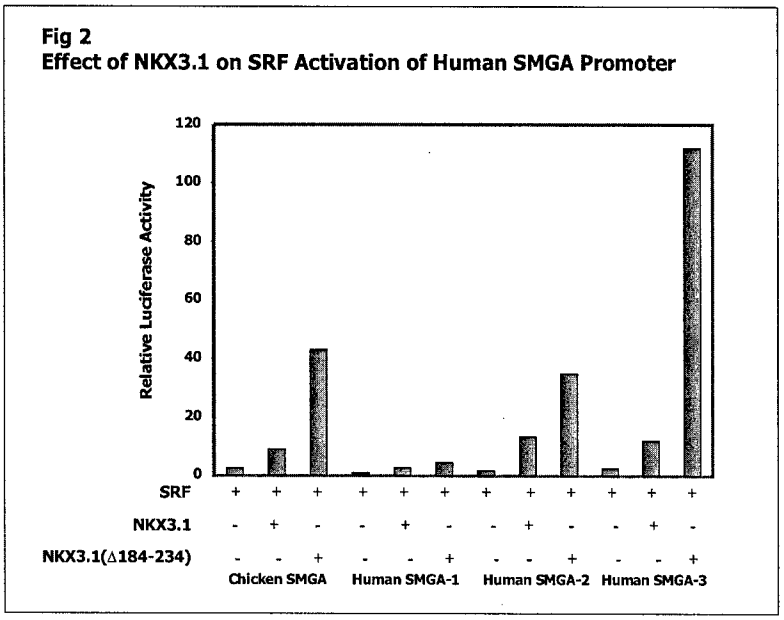
1. Testing of human NKX3.1 effects on chicken SMGA reporter plasmid.

The effects of human NKX3.1 on activity of the chicken smooth muscle γ-actin (SMGA) promoter region are shown in Figure 1. Similar to findings of Carson et al, with murine Nkx3.1, we showed that human NKX3.1 can enhance the effect of serum response factor (SRF) on the serum-response element containing SMGA promoter (5). We also showed that the human SMGA promoter is equally responsive to SRF and NKX3.1 (Figure 2).



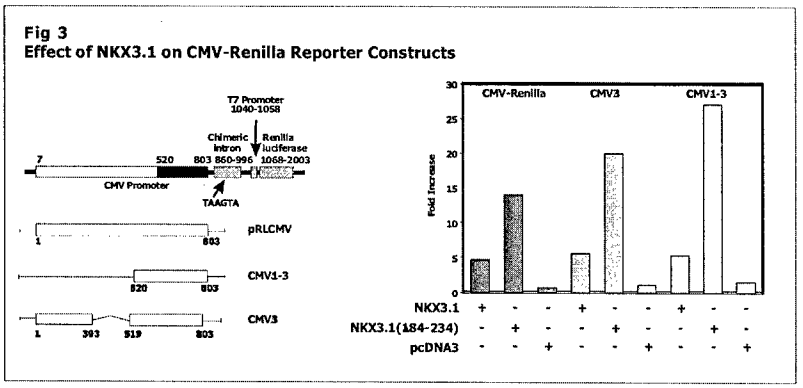
2. Construction of NKX3.1 and SRF expression vectors under control of HSVtk promoter.

As detailed in the year-1 Progress Report these constructs could not be engineered and this element of the Statement of Work was abandoned.



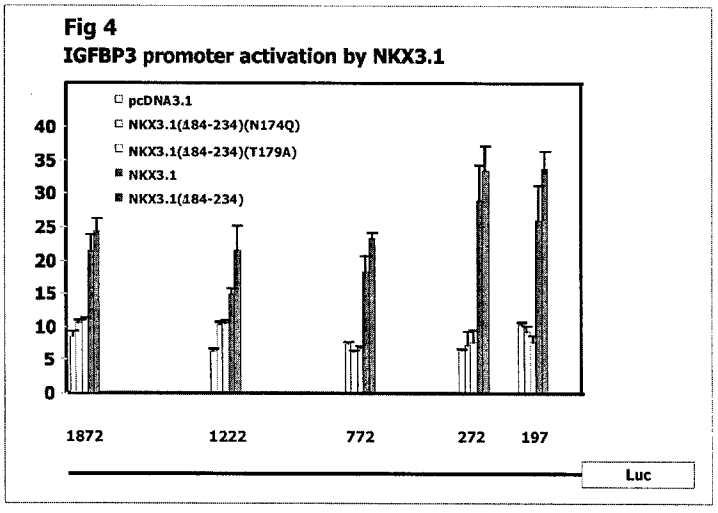
3. CMV Promoter Interaction

We found that NKX3.1 activates expression from the CMV promoter. Since this is a common promoter in eukaryotic expression constructs it has forced us to control all our reporter assays for the degree of NKX3.1 construct expression. We do so routinely and will not describe the data in detail at this time. Importantly, we have done deletion analysis of the CMV promoter and found that the 3' end is sensitive to transcriptional activation by C-terminal truncated NKX3.1, but not full length protein (Figure 3). There are no TAAGTA NKX3.1 recognition sites in the CMV promoter and mutation of the single binding site in the intron as shown does not affect the interaction with NKX3.1 or NKX3.1(Δ184-234). Therefore, this construct provides a qualitative assay for the activity of the C-terminal region and, we speculate, a transcriptional protein-protein interaction mediated by the truncated peptide.



3. IGFBP-3 Promoter Activation

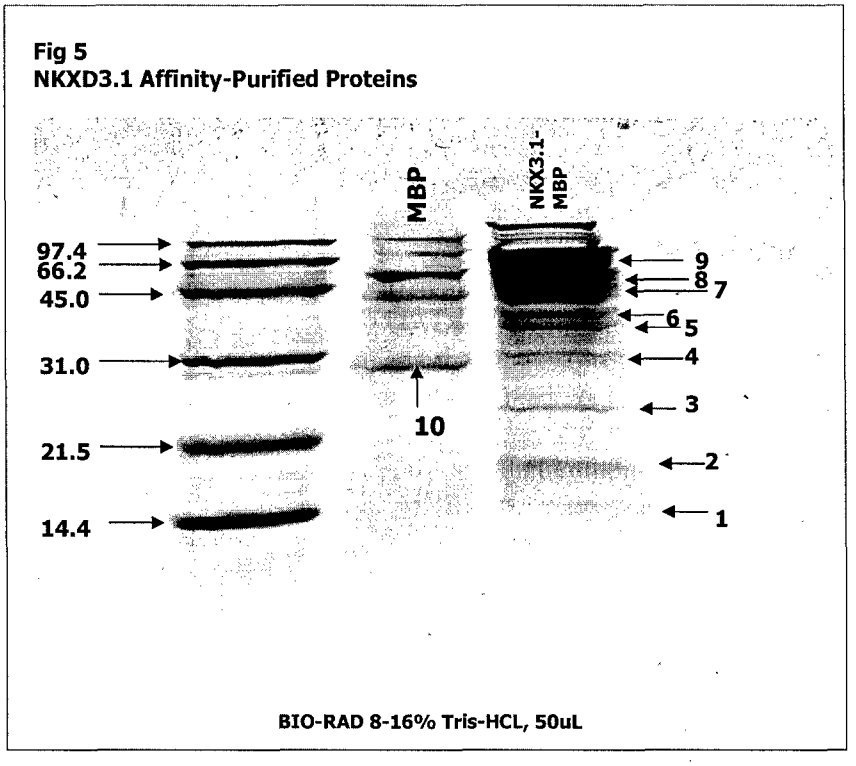
We found that NKX3.1 activates expression of *IGFBP3* mRNA and protein about 10-fold in PC-3 prostate cancer cells. These finding arose out of a cDNA microarray analysis comparing vector-transfected PC-3 cells with two clones transfected with an *NKX3.1* expression plasmid. Based on this interaction we found that a 197bp region of the proximal *IGFBP3* promoter is sensitive to NKX3.1 and NKX3.1(1-184), but not to two NKX3.1 proteins with mutations in the homeodomain that disrupt DNA binding (Figure 4). However, despite the sensitivity of the reporter assay to mutations that disrupt DNA binding, neither NKX3.1 nor NKX3.1(Δ184-234) bind to any region of the -197-0 *IGFBP3* DNA sequence. Therefore, this reporter activation is likely due to a protein-protein interaction with NKX3.1 that is dependent on the homeodomain.



B. Yeast-two-hybrid cloning of NKX3.1 binding partners. (Year 1-2)

1. Isolation of Y2H clones that bind WT NKX3.1
2. Sequencing clones and confirmation by GST pull-down
3. Construction of NKX3.1 R52C DB plasmid and selection of clones that bind to NKX3.1 R52C
4. Back selection of NKX3.1 R52C-binding clones against WT NKX3.1
5. Analysis by sequencing and GST pull-down of any clones that bind NKX3.1 R52C but not WT NKX3.1

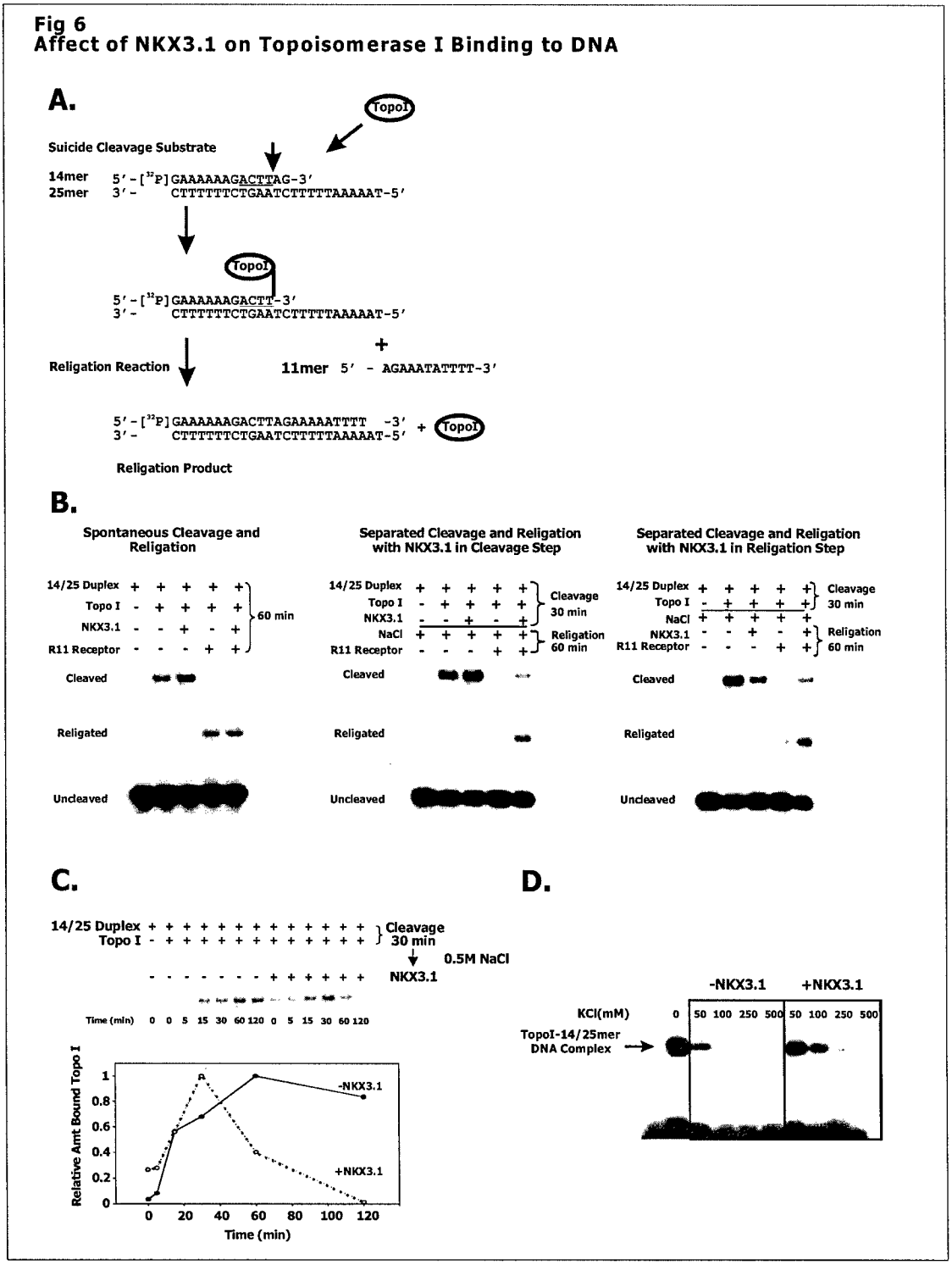
As described in the Year 1 Progress Report we had to abandon this element due to feasibility issues. The hybrid yeast two hybrid bait constructs – GAL4/NKX3.1 and VP-16/NKX3.1 were both biologically inactive presumably due to misfolding of the fusion proteins. Although we considered pursuing LEXA fusions that we used by C. Bieberich to identify PDEF as an NKX3.1 binding protein (6), after discussions with C. Bieberich we decided not to pursue these experiments. Instead we have decided to exploit affinity chromatography to isolate proteins that complex with NKX3.1. We made an affinity column of NKX3.1/maltose-binding protein (MBP) fusion protein as an affinity reagent. PC-3 cell extract was passed through the column and through a control MBP column. Polyacrylamide gel electrophoresis of the affinity-purified peptides that adhered to the two columns is shown in Figure 5. Note that the NKX3.1/MBP fusion protein selected many more proteins than the control MBP column.



Isolation of Topoisomerase I by NKX3.1 Affinity

To isolate cellular proteins that bind to NKX3.1 we passed a lysate of PC-3 prostate cancer cells through an affinity column made with a fusion protein of NKX3.1 and bacterial maltose-binding protein (MBP) bound to amylose beads. We have previously shown that the NKX3.1-MBP fusion protein is functional and can bind the high-affinity TAAGTA NKX3.1 DNA binding site (7, 8). NKX3.1-MBP adheres to an amylose column and can be eluted along with adherent proteins. As a control PC-3 cell extracts were passed through a parallel amylose column that was preloaded with electrophoretically pure MBP. Polyacrylamide gel electrophoresis of the proteins and the affinity

reagents that remain on the two columns after exhaustive washing were eluted and are shown in Figure 5. The NKX3.1-MBP fusion protein retained more proteins than the MBP column. A number of bands were subjected to in-gel digestion and the digestions were analyzed by nanoflow reversed-phase liquid chromatography coupled online with electrospray ionization tandem mass spectrometry. The indicated band was identified as human topoisomerase I. A band that migrated nearly in parallel from the MBP column did not contain peptide sequences of topoisomerase I. The approximate 70kDa migration of the topoisomerase I band is consistent with a known proteolytic cleavage fragment of the 91kDa full-length protein.



NKX3.1 Enhances Topoisomerase I Binding to and Cleavage of DNA, but not Religation

Topoisomerase I nicks DNA preferentially, but not exclusively, at 5'-(A/T)(G/C)(A/T)T-3'nucleotide sequence that lies at positions -4 to –1 relative to the cleavage site on the scissile DNA strand. Human topoisomerase I binds covalently to the –1 T nucleotide via tyrosine 723. DNA cleavage and religation by topoisomerase I can each be assayed in a "suicide cleavage" reaction using the oligonucleotide reagents shown in Figure 6A (9). The partial DNA duplex traps topoisomerase I in a complex because there is no substrate to initiate religation and enzyme release. Although the suicide complex is treated with proteinase K and denaturing reagents, a fragment of topoisomerase I remains covalently bound to the scissile DNA strand and migrates at a unique distance in a denaturing gel. Addition of the 11-mer acceptor provides a substrate for religation and release of topoisomerase I from the DNA duplex (10).

NKX3.1 enhanced the formation of the "suicide" complex (Fig 6B left panel lanes 1-3). NKX3.1 alone + 14/25mer in the absence of topoisomerase I showed no cleavage (not shown in this experiment but demonstrated in Figure 7). Addition of the 11-mer acceptor allowed religation and release of topoisomerase I to proceed (Fig 6B, left panel lanes 4-5).

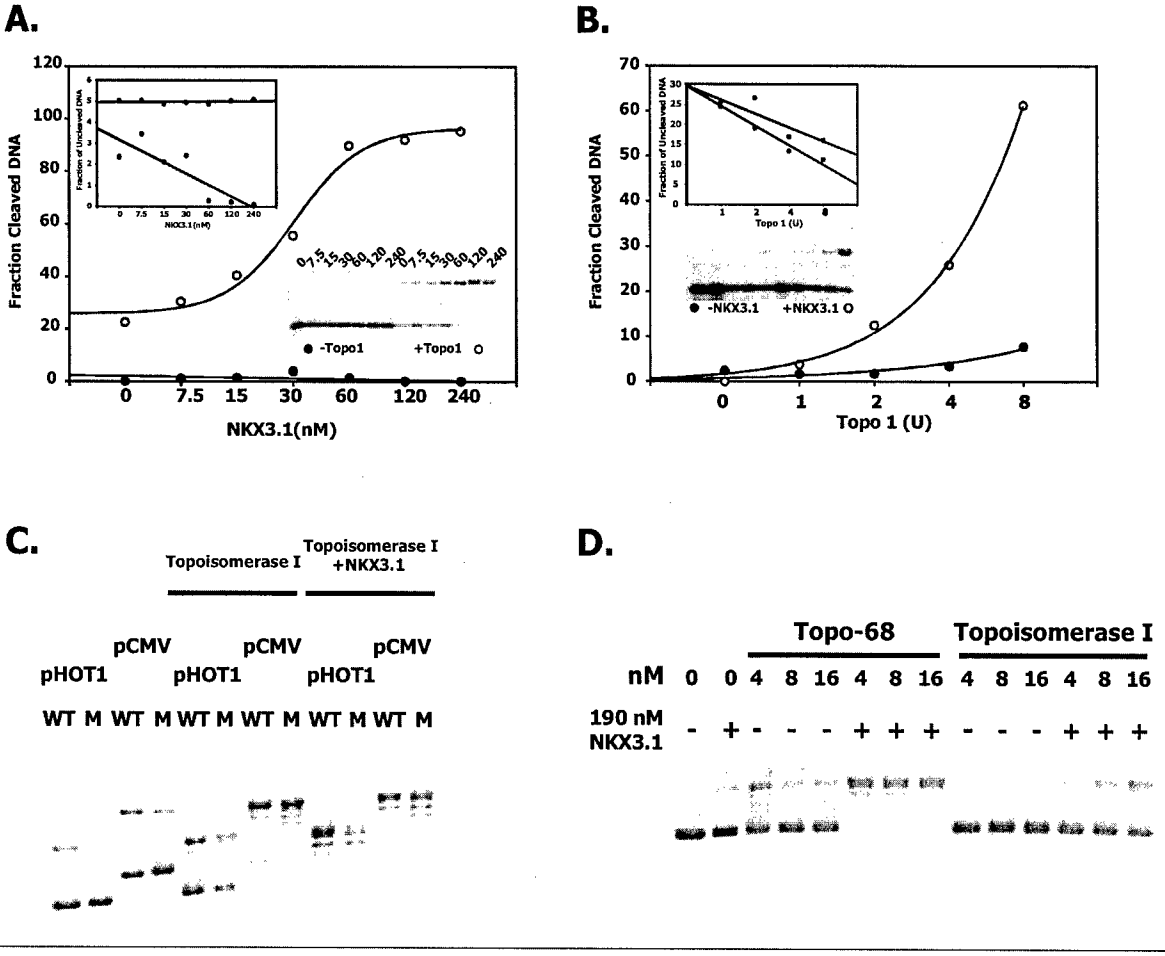
To discriminate cleavage and religation reactions, 0.5M NaCl was added to the reaction mixture to terminate the cleavage reaction. Higher salt concentrations cause dissociation of the topoisomerase I/DNA complex, but allow religation to proceed (11). NKX3.1 was applied either prior to or after the addition of 0.5M NaCl to determine its relative influence on formation of the topoisomerase I/DNA complex and DNA religation. When the topoisomerase I/DNA complex was allowed to form in the presence or absence of NKX3.1 and then arrested by addition of NaCl there was substantially more topoisomerase I/DNA complex in the presence of NKX3.1 than in its absence (Figure 6B lanes 2 and 3, center panel). However, once cleavage was arrested by the addition of NaCl religation proceeded at very similar rates as shown by the religation band in lanes 4 and 5 of Figure 6B center panel. Addition of the 11-mer religation acceptor allowed completion of the reaction but was not further enhanced by the presence of NKX3.1. This result suggests that NKX3.1 had an affect on cleavage, but not religation. When the topoisomerase I/DNA complex was arrested by addition of NaCl, addition of NKX3.1 had the unexpected effect of destabilizing the complex and allowing dissociation of topoisomerase I from its covalent bond with the partial duplex (Fig 6B, right panel lanes 2 and 3). If the 11-mer receptor was added at the same time as NKX3.1 there was more religation reflecting an effect of NKX3.1 on the completion of the DNA unwinding reaction (Figure 6B, right panel, lanes 4 and 5).

To examine further the effect of NKX3.1 on the topoisomerase I/DNA complex we examined the effect of NKX3.1 over time after the addition of 0.5M NaCl. The topoisomerase I/DNA continued to form at a slow rate up to 60min. in the presence of 0.5M NaCl. Between 60 and 120min. the complex was either stable or underwent dissociation to a small degree (Figure 6C). NKX3.1 accelerated the association of topoisomerase I and DNA, but also markedly accelerated the "off" rate, causing near complete dissociation of the covalent protein-DNA bond at 120min. (Figure 6C). Thus NKX3.1 alters the interaction of topoisomerase with its substrate and facilitates reversibility of the reaction in the absence of religation substrate. The forward reaction of topoisomerase I association with DNA was also enhanced by NKX3.1 as further shown by the formation of the duplex in the presence of increasing salt concentrations. NKX3.1 stabilized the topoisomerase I/DNA complex in the presence of higher salt concentrations (Figure 6D).

We next examined the kinetics of NKX3.1 interaction with topoisomerase I cleavage activity. The result is seen in Figure 7A where NKX3.1 alone neither cleaved nor bound the radiolabeled suicide substrate, but had a marked affect on topoisomerase I activity. The amount of topoisomerase I used generated little to no cleavage during the 30min reaction (Fig. 7A upper inset). The vertical axis in the Fig 7A inset indicates relative amount of uncleaved DNA determined by densitometry of the gel in the lower inset. We estimated the molar ratio at which the maximal effect of NKX3.1 was seen

at 10:1, NKX3.1:topoisomerase I. We also titrated topoisomerase I in the presence of 10nM NKX3.1 and demonstrated that NKX3.1 changed the rate and the efficiency of the cleavage reaction catalyzed by topoisomerase I (Figure 7B).

Fig 7
Affect of NKXe3.1 on Topoisomerase Activity

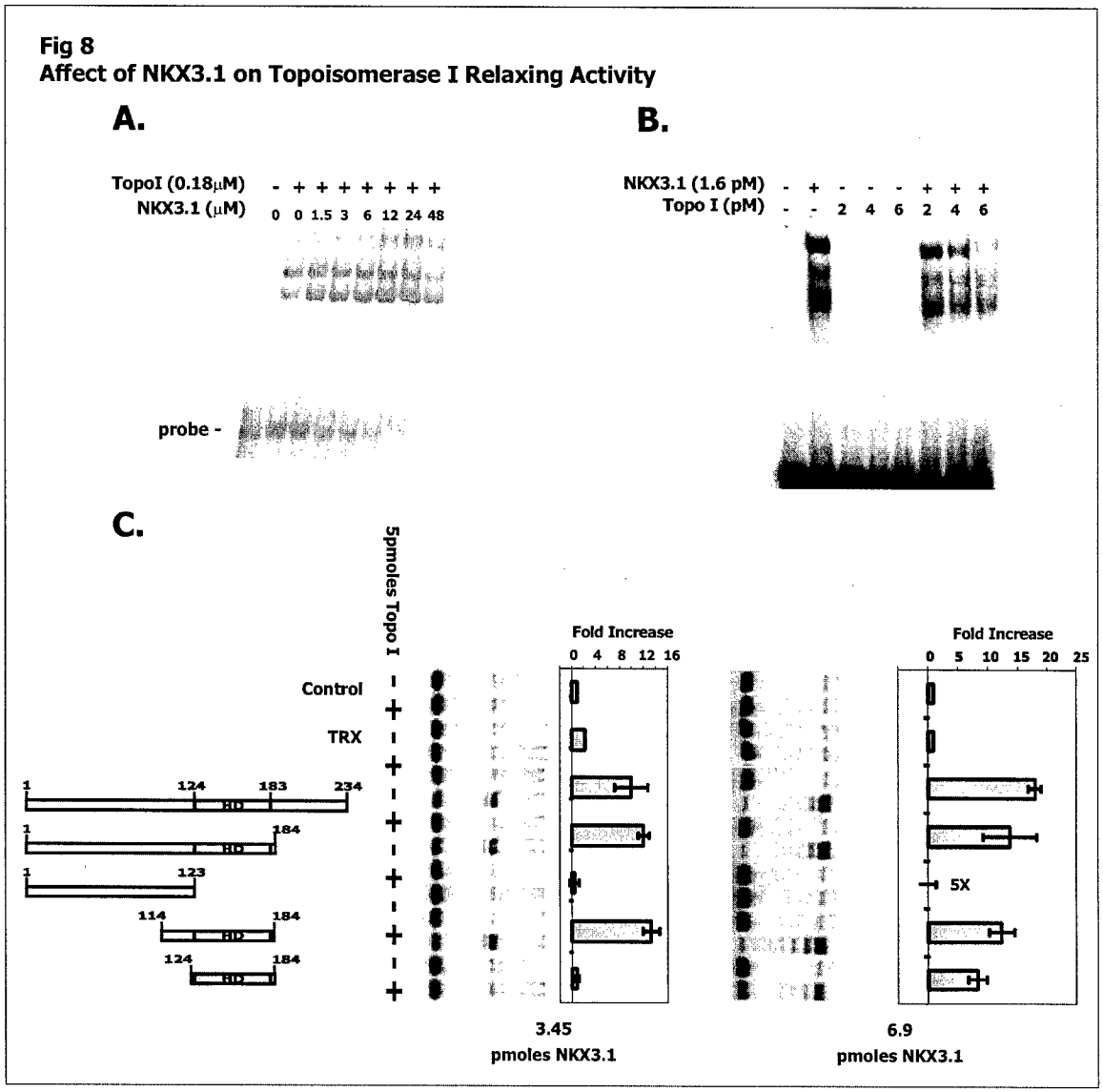


Topoisomerase I is also assayed by unwinding super coiled DNA often using a pUC12 plasmid that includes a 50 nucleotide insert derived from the *Tetrahymena* ribosomal RNA gene repeat as a substrate (pHOT1) (12). pHOT1 contains a single TAAGTC NKX3.1 hexanucleotide binding sequence. We mutated this sequence to a CAAATC that does not bind NKX3.1 (7) to control for possible NKX3.1 binding to the plasmid. In addition we used a plasmid pCMV that was constructed with pcDNA3 and *Renilla* luciferase. This plasmid also has a single TAAGTC site that was mutated as well. Both plasmids were unwound by topoisomerase I in a reaction that was enhanced by the presence of NKX3.1. Loss of the NKX3.1 binding sites on either of the plasmids had no effect on NKX3.1 interaction with the reaction (Figure 7C). Topoisomerase I contains an N-terminal domain important for nuclear localization and protein-protein interactions (13). The N-terminal domain is also a substrate for proteases and confers instability to topoisomerase in vitro. We compared the interaction of topo-68, an N-terminal (Δ1-190) truncated topoisomerase and full-length topoisomerase I with NKX3.1 (14, 15). This N-terminal deletion construct retained tryptophan 205, a residue critical for interactions with the loop domain, blunt-end ligation by topoisomerase I, and camptothecin sensitivity (16). We compared full-length topoisomerase I with topo-68 in a DNA

unwinding assay and found that topo-68 was activated by NKX3.1 perhaps even to a greater degree than full-length topoisomerase I (Figure 7D).

Binding of NKX3.1 and Topoisomerase I to DNA

NKX3.1 binds to topoisomerase I and can combine with the topoisomerase I/DNA complex. This interaction was suggested by an electromobility shift assay using the 50-mer *Tetrahymena* sequence as a probe and purified proteins. NKX3.1 alone did not bind to the *Tetrahymena* 50-mer probe. However, NKX3.1 enhanced topoisomerase binding as shown by the increasing intensity of the two species formed by topoisomerase I and the DNA probe and the depletion of the probe-alone band. Moreover, a super shifted complex can be seen suggesting the formation of a complex of probe, topoisomerase I, and NKX3.1 (Figure 8A). Binding of topoisomerase to NKX3.1 is of sufficiently high affinity to compete NKX3.1 from its cognate DNA sequence at a topoisomerase I:NKX3.1 molar ratio as low as 2:1 (Figure 8B). This competition suggested that topoisomerase I occupied the same binding site in the NKX3.1 homeodomain as the cognate DNA sequence. To confirm this observation we used purified recombinant NKX3.1 peptide fragments in a topoisomerase I relaxation assay with pHOT1 as a substrate (Figure 8C). In order to demonstrate the potentiating effect of NKX3.1 on topoisomerase I activity reaction conditions were used that resulted in minimal relaxation of 40pmoles super coiled pHOT1 by topoisomerase I alone. We used two concentrations of NKX3.1 and its peptide fragments with approximately 5pmoles of topoisomerase I. Under these conditions

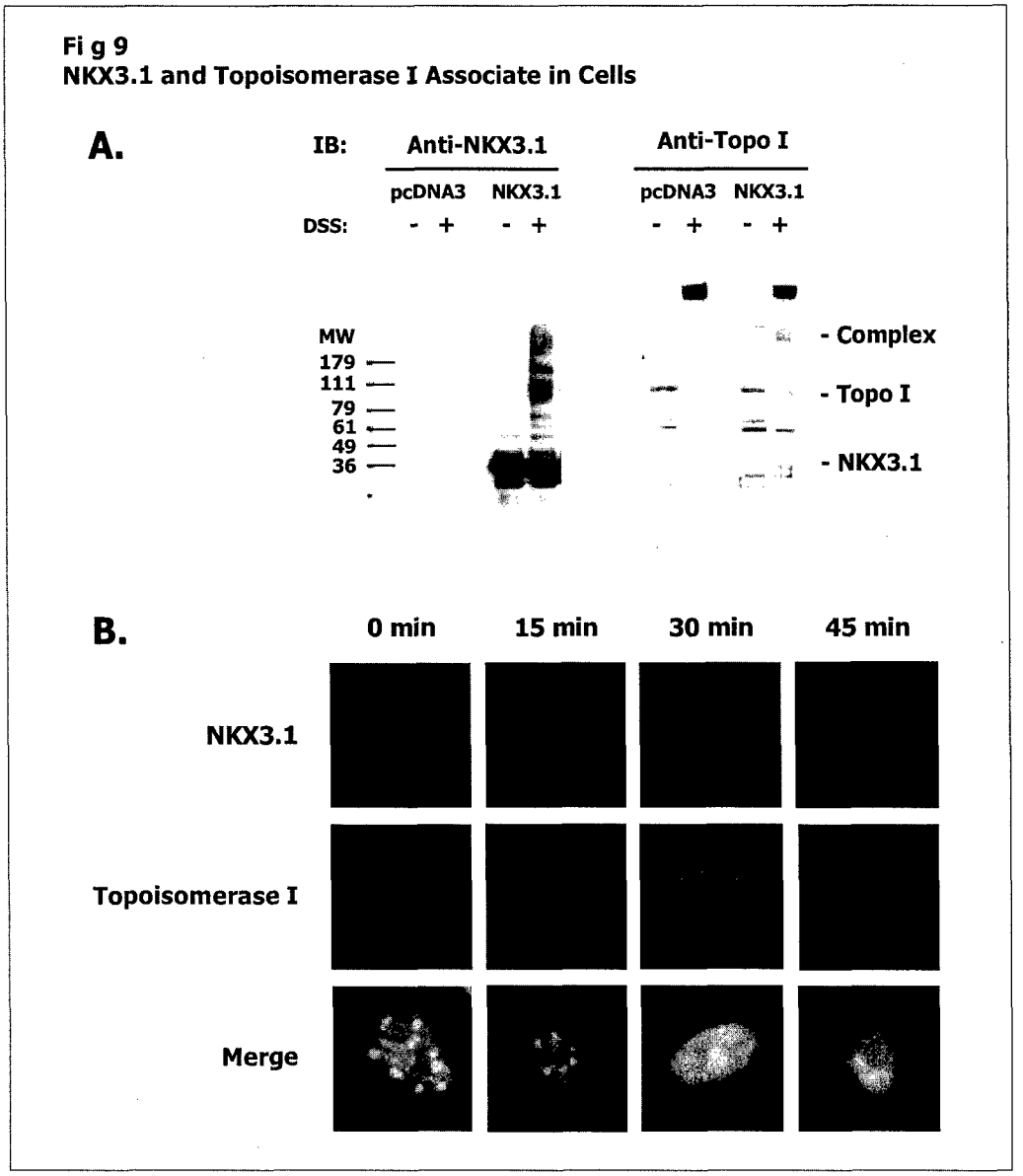


NKX3.1 potentiated DNA unwinding in a 30min reaction. Deletion of the inhibitory C-terminal domain distal to the homeodomain [NKX3.1(1-184)] had very little effect on the interaction with topoisomerase I. Deletion of the C-terminus and the homeodomain NKX3.1(1-123) abrogated the interaction with topoisomerase I, even at a molar ratio of NKX3.1:topoisomerase I of 7:1, suggesting that the homeodomain is critical for the interaction with topoisomerase I. The thioredoxin (TRX) tag alone had no effect on topoisomerase I activity and had no inhibitory effect when added to DNA relaxation assays with topoisomerase +/- NKX3.1 (data not shown). A DNA unwinding assay with either 3.8pmoles or 19pmoles electrophoretically pure NKX3.1(1-123) without the TRX tag was compared to 3.8pmoles NKX3.1(1-184) and showed no detectable effect of NKX3.1(1-123) on topoisomerase I DNA unwinding activity (data not shown). We also repeated the experiment with NKX3.1(1-123) cleaved from the TRX tag and confirmed the near absence of interaction with topoisomerase I unwinding activity.

NKX3.1(114-184) containing the homeodomain and upstream 10 amino acids showed comparable activity to the full-length protein. Interestingly, the 10 amino acids N-terminal to the homeodomain were important to optimize the interaction since the homeodomain alone [NKX3.1(124-184)] was inactive at a ~3:5 molar ratio, but active at a ~7:5 molar ratio with topoisomerase I.

NKX3.1 and Topoisomerase I Interact in Vivo

To demonstrate that topoisomerase I complexed with NKX3.1 in vivo, we transfected 293T cells with either an empty pcDNA3 vector or the vector containing *NKX3.1* and generated protein extracts that were treated with the cross linking reagent disuccinimidyl suberate (DSS). We subjected the lysates to western blotting with antibodies to both NKX3.1 and topoisomerase I. Only the presence of both NKX3.1 expression and treatment with DSS resulted in a complex of the correct mass that was seen well with the antibody to topoisomerase I, but less well when the blot was probed with NKX3.1 antiserum (Figure 9A). To further confirm that NKX3.1 and topoisomerase I interact in cells we performed a colocalization experiment in LNCaP prostate cancer cells and visualized only endogenous proteins. Topoisomerase I and NKX3.1 were both seen to reside in nuclear bodies. When cells were exposed to γ-irradiation both topoisomerase I and NKX3.1 continued to be colocalized in the nucleus, but in a more diffused configuration that was seen within 45 minutes of exposure to γ-irradiation (Figure 9B).

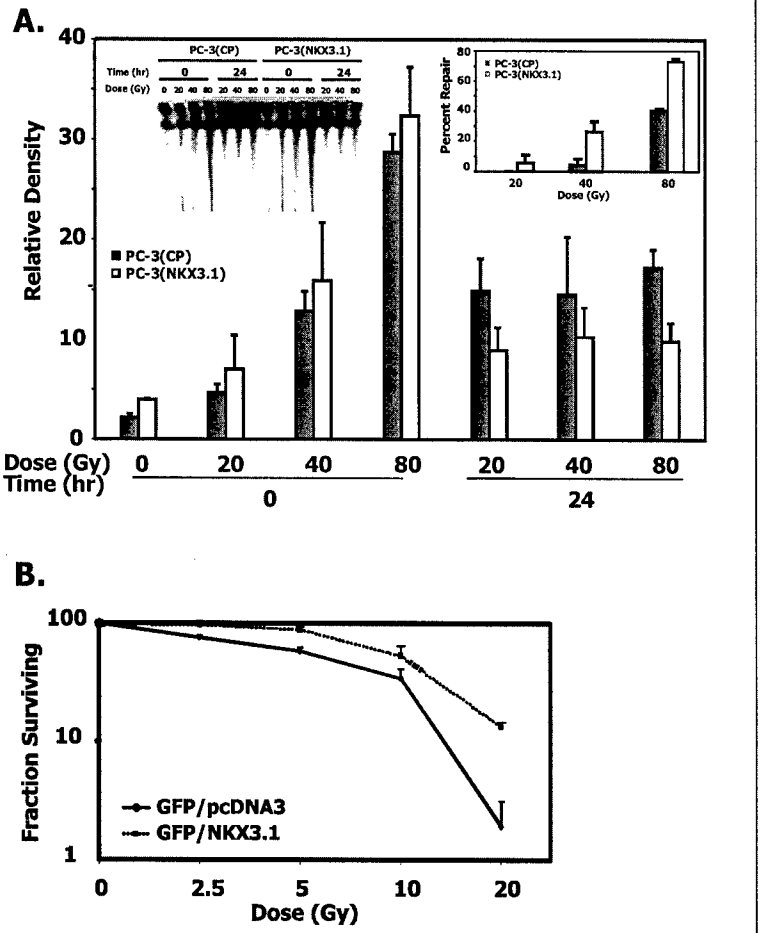


NKX3.1 and Topoisomerase I Interaction in DNA Repair

NKX3.1 is a tumor suppressor, but how it interacts with oncogenic pathways has not been established. Because of the interaction with topoisomerase I which may be involved with DNA repair mechanisms, we sought to determine if the expression of NKX3.1 affected DNA repair and cell survival after DNA damage. We used γ-irradiation as a quantitative and reproducible cause of DNA damage with the understanding that the predominant resulting lesions are single- and double-strand breaks. We irradiated PC-3 prostate cancer cells stably transfected with an NKX3.1 expression vector. PC-3 cells express no detectable NKX3.1 and transfectants express about 25% of the levels seen in LNCaP cells treated with R1881. Compared to control transfected cells, NKX3.1 expressing cells displayed more DNA repair 24hr after exposure to γ-irradiation. DNA damage and repair was measured by quantitating lower molecular weight DNA fragments migrating in pulsed-field gel electrophoresis. In the presence of NKX3.1 there was slightly greater amounts of lower molecular weight DNA species in the transfected cells as well as all the exposure cohorts at t=0. However, at 24h there was 50% more DNA repair in the NKX3.1-expressing cells than in the control PC-3 cells (Figure 10A).

The effect of NKX3.1 expression on DNA repair furthermore translated into survival after exposure to γ-irradiation. PC-3 cells were subjected to transient transfection with a GFP expression vector and either an empty vector, pcDNA3, or NKX3.1 expression plasmid. Cells were sorted for GFP expression, irradiated, and plated for clonogenic survival. Cells transfected with NKX3.1+GFP demonstrated improved survival after exposure to γ-irradiation compared to cells transfected with GFP alone further supporting the notion that NKX3.1 expression in prostate cells improves DNA repair efficiency (Figure 10B).

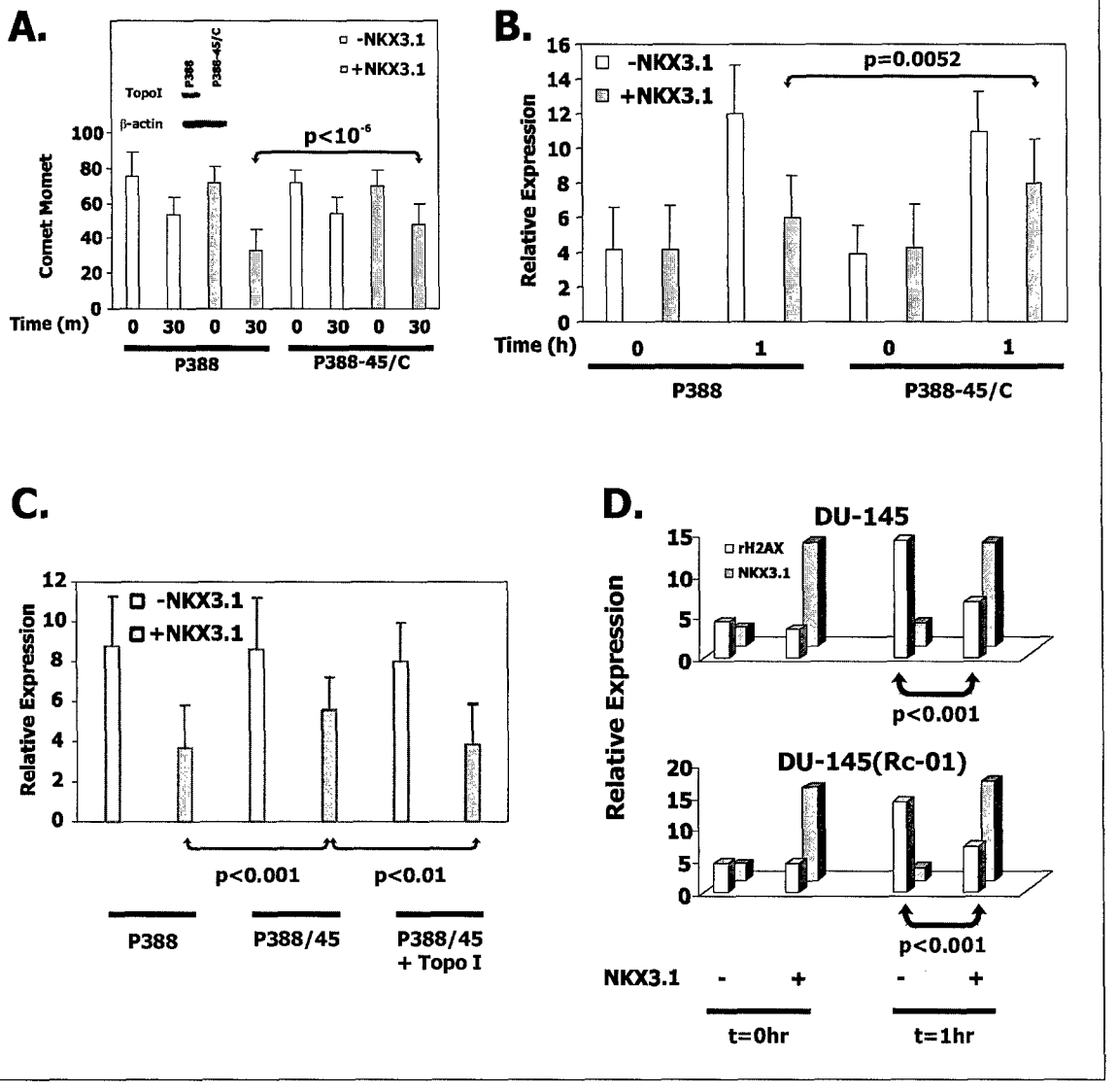
Fig 10
Effect of NKX3.1 on DNA Repair



We next determined that the effect of NKX3.1 on DNA repair was due, at least in part, to interaction with topoisomerase I. We used P388 murine leukemia cells and a derivative camptothecin-resistant line P388-45/C that expresses no functional topoisomerase I as confirmed by western blot shown as an inset in Figure 11A (17). We first analyzed individual cells transfected with GFP ± NKX3.1 in a comet assay by quantitating the comet "tail" at 0 and 30min as an index of DNA repair. Cells were identified by GFP expression and analyzed for the area of the tail normalized to the nuclear area (comet moment). Expression of NKX3.1 had a significant effect on DNA repair in P388 cells, but essentially no effect in P388-45/C cells. The difference in DNA repair between the two cell lines transfected with NKX3.1 was highly significant, $p<10^{-6}$ (Figure 11A).

We used a second assay for DNA damage by staining cells for phosphorylated histone γH2AX as an index of DNA damage in transfected and sorted P388 and P388-45/C cells (18). There was substantially more phosphorylated histone γH2AX at 1h after irradiation in the topoisomerase I-deficient cells than the parental cells ($p<0.0052$) (Figure 11B), confirming the findings of the comet assay. The effect of NKX3.1 on levels of phosphorylated histone γH2AX that was deficient in the P388-45/C cells could be restored to baseline by transfection with exogenous topoisomerase I, underscoring the role of topoisomerase I on the effect of NKX3.1 on DNA repair (Figure 11C). Lastly we tested a second cell line that had also been selected for resistance to camptothecin. DU-145(Rc-01) cells are a derivative of DU-145 prostate cancer cells that have acquired a point mutation in topoisomerase I (R364H) rendering them camptothecin resistant, but retaining topoisomerase I activity (19). In these camptothecin-resistant, but topoisomerase I-intact cells there was no difference in the effect of NKX3.1 on phosphorylated histone γH2AX between the parental and derivative cell lines (Figure 11D).

Fig 11
NKX3.1 and Topoisomerase I Mediate DNA Repair



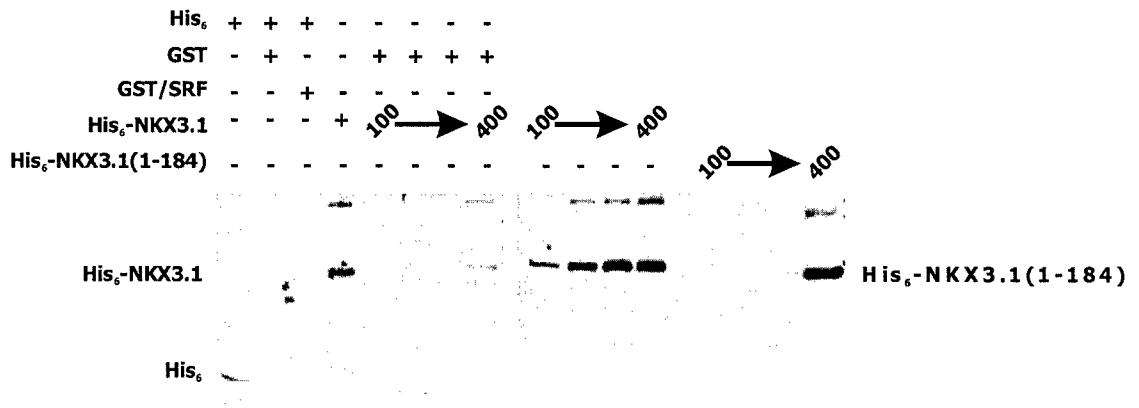
C. Structural analysis of NKX3.1 – regions necessary for protein/protein interactions. (Year 3)

1. Construct GAL-4-DB vectors with *NKX3.1* deletion constructs.
2. Y2H analysis of deletion mutants binding to GAL4-AD-SRF and other protein binding partners.
3. GST pull down assay using deletion mutants of NKX3.1-GST fusion protein and SRF or other cloned proteins
4. SMGA reporter gene analysis of transcriptional activation by *NKX3.1* deletion mutants and SRF. CMV reporter gene analysis of *NKX3.1* deletion mutants cotransfected with clones isolated in aim 2.

This element of the Statement of Work is addressed in part by the data shown in Figure 8C above. We have also developed a GST-pulldown assay to analyzed physical association between NKX3.1 and other proteins. To develop this assay we used full-length SRF cloned into pGEX-6P3, GST-SRF fusion protein, and GST all expressed in BL21-Gold (DE3) *E. coli* (Stratagene). Proteins were bound to glutathione-sepharose beads (Amersham Pharmacia). Equal amounts of bound GST fusion proteins were incubated with pure recombinant His_6-NKX3.1 FL, His_6-NKX3.1(1-184) or His_6 in PBS for 1

hour. Where indicated the protein concentrations varied from 100pM to 400pM in 100pM increments. The beads were washed four times with PBS and resuspended in 2X SDS sample buffer. Samples were subjected to SDS-PAGE and western blotting using an anti His_5 antibody (Qiagen). We see that there is a small background binding of His_6-NKX3.1 to GST, but that the GST/SRF fusion protein binds much more strongly. His_6 does not complex with GST or GST/SRF and therefore is unlikely to mediate nonspecific binding between His_6-NKX3.1 and SRF. Reduced, but evident binding is seen with His_6-NKX3.1(1-184) in contrast to the 6-fold enhanced coactivation activity of the C-terminal truncated protein. Therefore, the transcriptional interaction between NKX3.1 and SRF requires DNA as a binding mediator and is not quantitatively related to the interaction of the two

Fig 12
GST Pulldown Assay with SRF and NKX3.1



proteins alone.

Key Research Accomplishments

1. Identification topoisomerase I as a target for NKX3.1 binding.
2. Modulation of topoisomerase enzymatic activity by NKX3.1.
3. Demonstration of a role for NKX3.1 and topoisomerase I in DNA repair.
4. Establishment of a GST-pulldown assay for SRF and NKX3.1.

Reportable Outcomes

Same as key accomplishments. This work was presented, in part, at the 2004 Annual Meeting of the American Association of Cancer Research. The data are currently described in a manuscript that is submitted for publication.

Conclusions

1. NKX3.1 binds topoisomerase I in the absence of DNA.
2. NKX3.1 enhances topoisomerase I cleavage of DNA either by facilitating DNA binding or by accelerating DNA cleavage.
3. NKX3.1 and topoisomerase I cooperate to mediate repair of γ-irradiation-induced DNA damage.

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